VACUUM OR MODIFIED ATMOSPHERE PACKAGING FOR REFRIGERATED RAW FISHERY PRODUCTS

NATIONAL ADVISORY COMMITTEE ON
MICROBIOLOGICAL CRITERIA FOR FOODS

Adopted March 20, 1992

EXECUTIVE SUMMARY

The National Advisory Committee on Microbiological Criteria for Foods (Committee) examined microbiological safety issues associated with vacuum or modified atmosphere packaging (VAC/MAP) of refrigerated raw fishery products.

The Committee found that the primary preventive measure against the Clostridium botulinum hazard in these products is the temperature control at or below 38°F (3.3°C) from packaging through preparation. Other microbiological concerns such as Yersinia enterocolitica, Listeria monocytogenes, and histamine production can also be effectively addressed by low temperature storage.

Secondary preventive measures to further reduce hazards were also evaluated.

The Committee recommends that VAC/MAP technology be permitted for raw fishery products only when the following conditions are met:

- The products are packaged under an established Hazard Analysis Critical Control Point (HACCP) plan.
- Detectable spoilage and rejection by the consumer precedes the possibility of toxin production.
- High quality raw fish is used.
- Packaged product is stored at or below 38°F (3.3°C).
- Product is adequately labeled for storage temperature, shelflife, and cooking requirements.

The Committee also recommends minimum conditions for VAC/MAP technology, including protocols for inoculated pack studies, needs for sensory and statistical evaluation, use of secondary preventive measures, and additional research priorities that will facilitate rational development and safe use of VAC/MAP technology.

INTRODUCTION

Vacuum or modified atmosphere packaging (VAC/MAP) of refrigerated fishery products can present unique food safety problems. Historically, the principal safety concerns are related to low temperature outgrowth and toxin production by nonproteolytic types B, E, and F Clostridium botulinum which are commonly associated with fishery products. There are reports within the scientific literature for some fish species that botulinum toxin production can occur in fish before it would be rejected by consumers because of obvious spoilage.

In 1985, the National Research Council, a subcommittee of the National Academy of Sciences (NAS), concluded in a report entitled An Evaluation of Microbiological Criteria for Foods and Food Ingredients that "Thorough studies are needed to evaluate the potential hazard of refrigerated storage of raw fish in vacuum packages and in modified gaseous atmospheres. Until such time that the safety of this storage method for raw fish is validated, this practice is not recommended by this subcommittee because of its potential health risks."

Since publication of the NAS report, interest in VAC/MAP technology in seafood processing has increased. The increased interest in VAC/MAP technology for raw fish could well be predicted since it extends shelflife and allows penetration of new markets distant from coastal areas. This technology has received widespread use for raw fish products throughout Europe where food distribution systems and shopping patterns are different from those in the United States Regulatory agencies in the United States have not taken a uniform position on the application of VAC/MAP technology to raw fish processing and marketing operations.

Recognizing the interest in VAC/MAP technology and the associated safety concerns, the National Advisory Committee on Microbiological Criteria for Foods (Committee) elected to review the microbiological issues associated with product safety concerns. The nature of the review was to determine if new data or information had been developed since the 1985 NAS recommendation against the use of the technology for raw fish products. The review process employed by the Committee was to evaluate regulatory activities relative to this technology and to review the literature pertaining to the microbiological risks. The literature review was grouped into the following four categories: general review articles; C. botulinum; other potential microbial hazards, e.g., Listeria, Yersinia, Aeromonas, and histamine formation; and antimicrobial treatments.

GENERAL FINDINGS

1. Currently, vacuum or modified atmosphere packaged (VAC/MAP) fishery products are held at refrigeration temperatures during wholesale distribution and at retail.

2. Regulatory requirements for VAC/MAP are limited. The U.S. Department of Commerce (USDC), through its voluntary seafood inspection program, requires process approval and, in some cases, Clostridium botulinum inoculated pack studies.

3. The U.S. Food and Drug Administration (FDA) is reviewing specific seafood safety issues associated with VAC/MAP technology applied to refrigerated fishery products. The FDA has not officially addressed the use of VAC/MAP technology at the retail level through an interpretation of its retail model code. The Association of Food and Drug Officials (AFDO) advises against using VAC/MAP technology on fish at the retail level.

4. Much of the literature on the use of VAC/MAP technology for fishery products details the extension of refrigerated shelflife or quality attributes but not the issue of product safety.

5. For studies that investigated microbiological safety, C. botulinum outgrowth and toxin production were demonstrated in some inoculated packs. However, experimental designs of these studies have varied widely. Variables include species of fish, inoculum type, inoculum size, method of inoculation, storage times and temperatures, gas composition, additives, and analyses for product decomposition and toxin production. Because of the conflicting results from these studies, it is not possible to draw definitive conclusions regarding the safety of this packaging technology applied to refrigerated raw fishery products.

6. Clostridium botulinum is found in both freshwater and saltwater species of fish. The prevalence of C. botulinum is widespread, but its incidence is low. The numbers in fish flesh, when detected, are very low, ($\leq$ 10 spores/g), with most reports indicating $\leq$ 1 spore/g. Often those C. botulinum isolated are proteolytic types A, B, and D which cannot grow at refrigeration temperatures. The psychrotrophic, non-proteolytic strains are isolated less frequently.

7. Literature revealed no C. botulinum toxin production in uninoculated pack studies and in very few uninoculated controls from other research. Some of the inoculated pack studies used inoculum sizes of 10^4 - 10^6 spores/g. These inoculum sizes appear unrealistically high for purposes of evaluating risk even under "worst case scenarios."

8. Clostridium botulinum does not grow below 38°F (3.3°C) and only very slowly between 38 and 40°F. Storage times and temperatures ranged widely among the studies reviewed. Some inoculated pack studies used high incubation temperatures of 59, 68 and 86°F (15, 20, and 30°C). These studies are not germane to the Committee's consideration. Some inoculated pack studies were conducted at refrigeration temperatures. Fish inoculated with high numbers of spores have been shown to become toxic after 6-8 days as temperatures increase to 50°F. Therefore, storage temperatures of 40-50°F and temperatures which fluctuate in that range and even higher are a cause for concern. These product temperatures can be found during distribution and retail storage. Extended storage under these conditions is hazardous.

9. Other psychrotrophic organisms (Yersinia enterocolitica, Listeria monocytogenes and Aeromonas hydrophila) were inhibited by CO_2 or by vacuum to varying degrees. However, the most important factor for their control was a temperature low enough to inhibit psychrotrophic C. botulinum. Low temperatures also effectively inhibited histamine producers, irrespective of the gaseous composition used. Histamine was produced primarily by aerobic and mesophilic microorganisms.

10. There is no consensus on secondary measures to provide an extra margin of safety. One such measure being used is the USDC requirement for a "use by" date on the product not to exceed 10 days from the date of pack.

11. Food additives such as salt, sorbates, nisin, phosphates, ethylenediaminetetraacetic acid (EDTA), and ascorbates have been studied for their ability to inhibit the growth of C. botulinum in raw fish. The results are inconclusive and more research is required before these additives may be considered as effective secondary preventive measures.

12. Irradiation has been shown to inhibit the growth of C. botulinum in VAC/MAP raw fishery products. Doses of irradiation high enough to inactivate C. botulinum spores result in organoleptically unacceptable seafood. Low dose irradiation further extends the shelflife of VAC/MAP fish at refrigeration temperatures, but does not inactivate spores.

13. Stochastic (predictive) modeling for risk assessment in the study of C. botulinum in fish species under VAC/MAP appeared in several articles. For example, shelflife linear and logistic models were developed to predict the probability of one spore initiating growth and toxin production by a particular day and at a particular temperature of storage for various fish species.

RECOMMENDATIONS

Based on these findings, the Committee recommends that:

1. The unrestricted use of vacuum or modified atmosphere packaging (VAC/MAP) technology for refrigerated raw fishery products not be permitted.

2. The restricted use of VAC/MAP for refrigerated raw fishery products can be considered only if sufficient safeguards detailed in an established Hazard Analysis Critical Control Point (HACCP) plan can be implemented and verified to control the specific safety concerns described elsewhere in this report.

3. VAC/MAP technology be permitted only when it is assured that detectable spoilage and rejection by the consumer precedes the possibility of toxin production.

4. VAC/MAP should be used only with high quality raw fish. It must not be used to extend the shelflife of fish whose quality has deteriorated.

5. Research should be conducted to define the minimum conditions for control, incorporating reasonable limits for inoculation size, storage temperatures, and stochastic (predictive) modeling techniques.

6. VAC/MAP products must be held at or below 38°F (3.3°C) at all points from packaging through final preparation. This temperature requirement must be clearly indicated on shipping cartons and retail package labels.

7. Secondary measures in addition to refrigeration must be employed to increase assurance of product safety. These include additional labeling requirements and stringent processing controls.

8. VAC/MAP technology must ensure that the intended vacuum or intended MAP gas compositions are achieved with appropriate films.

9. The minimum conditions for VAC/MAP technology as described below must be followed.

MINIMUM CONDITIONS FOR VAC/MAP TECHNOLOGY CONTROL

1. Raw Fish Quality

Prior to packaging, proper handling of raw fish must be assured from the point of harvest. Vacuum or modified atmosphere packaging (VAC/MAP) must not be used to extend the shelflife of fish whose quality has deteriorated.

2. Hazard Analysis Critical Control Point Plan

A Hazard Analysis Critical Control Point (HACCP) plan from point of packaging through retail sale must be developed for VAC/MAP, recognizing that rigid temperature control is the primary preventive measure to ensure safety.

3. Hazard Analysis/Risk Assessment

The studies to support hazard analysis/risk assessment must be completed by food safety experts who are competent in HACCP systems, Clostridium botulinum methodology, sensory evaluations, and statistical procedures.

The evaluated system cannot be used for marketing fish unless both the experimental results and the statistical modeling demonstrate that odor rejection will always precede toxin production. If this is not demonstrated, i.e., if toxin production precedes odor rejection, VAC/MAP technology cannot be used for the species of fish studied.

No new study to demonstrate the efficacy and safety of the process is necessary as long as the data from the basic study can be applied. A new study, including the inoculated pack and shelflife, however, is necessary when different species of fish are used, a new ingredient is added, a different gas composition is proposed, or the distribution systems or targeted users have substantially changed.

The studies to support hazard analysis/risk assessment of VAC/MAP include the following:

A. Process Description

A HACCP-based description of the species of fish investigated, the handling procedures and the gas composition must be developed.

B. Inoculated Pack Studies

Inoculated pack studies must be performed for each type of fish and packaging technology to confirm the efficacy and safety and to guide the development of an appropriate

distribution strategy. All studies must be conducted in a facility that has the safeguards in place to work with C. botulinum. Inoculation studies with C. botulinum must be performed in accordance with the procedure in Appendix 1.

C. Sensory Evaluation

A sensory evaluation method using specific odor rejection criteria to determine the onset of spoilage must be selected, standardized, and accepted.

D. Statistical Evaluation

Stochastic (predictive) statistical models must be based on the experimental data to evaluate the potential of toxin production before odor rejection according to the principles described in Appendix 2.

4. System Design Validation

Use the results of the inoculated pack and sensory evaluation study to determine whether the system design is effective and safe. Modify the processing system where necessary. Perform additional inoculated pack and sensory evaluation studies if the system is modified. Validate the HACCP plan. The total processing system and associated HACCP system must be approved by food safety experts competent in assessing the potential food safety hazards.

5. Labeling Requirements

Labeling requirements must include:

A. "KEEP REFRIGERATED AT 38°F (3.3°C) OR BELOW"

B. "USE BY DATE"

C. "COOK FULLY BEFORE SERVING"

The use-by date must be experimentally determined as referenced in Appendix I and should be a date at which product quality is still maintained and shown to be well in advance of possible toxin production.

6. Time and Temperature Records

Time/temperature recorders should be enclosed in selected sealed master cartons during shipment of seafood products. At the time each shipment is received, the individual time/temperature charts should be examined and the shipment or carton rejected if the product temperature exceeds 38°F.

Technology, reliability, and costs of time/temperature integrators have been developing to the point where their use is becoming very practical. The Committee has previously recommended the use of such time/temperature integrators on individual retail packages where this technology has been shown to be effective and economically feasible.

Procedures for Nonproteolytic *Clostridium botulinum* Inoculated Pack Studies to Evaluate the Potential Risk of Vacuum or Modified Atmosphere Packaging of Refrigerated Raw Fishery Products

Clostridium botulinum inoculated pack studies for raw fish are done to identify the risk of botulinal toxin production in products held at refrigeration temperatures above 38°F (3.3°C). It is important to relate time of overt spoilage (i.e., unfit for human consumption) to time of botulinal toxin production.

Considering the extreme toxicity of botulinal toxin, it is imperative that *C. botulinum* inoculated pack studies be designed and conducted only by experts who understand the hazards of botulinal toxin and are familiar with proper safety precautions for handling *C. botulinum*. Several points should be considered in designing a *C. botulinum* challenge study. Examples include:

1. Types and number of strains of *C. botulinum* to be used.
2. Methods for spore production, preparation, and enumeration.
3. Number of spores to be inoculated.
4. Methods for inoculating product with spores.
5. Packaging of product.
6. Time(s) and temperature(s) of product incubation.
7. Sample size, sampling times, number of samples to test.
8. Botulinal toxin testing procedure.
9. Product analyses to be performed during the study.

A. Types and Number of *C. botulinum* Strains

Nonproteolytic types of *C. botulinum* are used in challenge studies. A mixture of a minimum of five strains of nonproteolytic types B and E, and one strain of nonproteolytic type F is suggested. Strains should be selected from as many diferent sources as possible and may include:

Nonproteolytic Type B	Nonproteolytic Type E	Nonproteolytic Type F
2B	Beluga	83
17B	Saratoga	187
2129B	Minnesota	202
17844B	Iwanii	3194
KAPI-B	Alaska	
25765B	Birmingham	
	070	
	G21-5	

Strains 17B and Beluga should be used in all studies.

Each strain of C. botulinum used for inoculated pack studies periodically (yearly) should be assayed by the mouse bioassay for toxin production. Any culture producing less than 1000 MLD/ml should not be used; either a productive culture of the same strain should be obtained or a different strain should be used in lieu of the nonproductive culture.

B. Methods for Spore Production, Preparation, and Enumeration

Spore crops may be produced by a variety of methods. Examples include: (1) the use of many different sporulation broths that are selected on the basis of strain sporulation characteristics; (2) biphasic methods using different types of liquid media over different types of agar media; and (3) agar media (such as anaerobic egg yolk agar) held under anaerobic conditions. There appears to be no common approach to producing spore crops. Most investigators use an incubation temperature for nonproteolytic strains of 26 to 30-,36°C. Time to sporulation depends on the cultural conditions and the strain of C. botulinum, and is determined by periodic microscopic examination of the culture. The best spore production method for nonproteolytic strains is in liquid medium (e.g., TPGY medium: trypticase 5%, peptone 0.5%, yeast extract 2%, dextrose 0.4%, and sodium thioglycollate 0.1%) with slight variation in dextrose content (from 0.1-0.4%) and incubated at 26 to 30/36°C for 10 to 12 days.

During harvesting, spores should be washed three times with sterile distilled water and appropriate centrifugation. The spore suspension in sterile distilled water should be stored in vials at refrigeration temperatures at or below 38°F (3.3°C).

Spores may be enumerated by a 3 or 5-tube most probable number (MPN) procedure. Dilutions of non-heat-shocked spores (or product) are inoculated into tubes of TPGY medium and the TPGY tubes are incubated anaerobically in Gas-Pak jars at 30°C for 7 days (with subsequent trypsinization if the mouse bioassay for toxin detection is used). Pure spore suspensions can be enumerated by visually examining tubes for growth; however, for food samples which contain other organisms, the mouse test or an equivalent assay must be used to confirm the presence of botulinal toxin.

Alternatively, spore crops with sufficient numbers of spores can be enumerated by direct plating procedures. Nonheat-shocked spores are diluted and plated onto anaerobic egg yolk agar or an equivalent medium held at 26 to 30°C for 48-72 hours under anaerobic conditions.

C. Number of Spores Inoculated

Spore mixtures to be used in inocula should contain an approximately equivalent number of spores of each strain of C. botulinum in the cocktail. Spores should be diluted appropriately in sterile distilled water and stored at refrigeration temperatures at or below 38°F (3.3°C).

Raw fish require surface inoculation. Since spores cannot be uniformly distributed throughout, an inoculum level of 10 to 100 spores per gram is recommended.

D. Methods of Inoculation

Only high quality raw fish should be used in inoculation studies. Samples should be surface inoculated by dropwise (up to 0.1 ml per drop) addition of inoculum that is spread out in a thin layer using sterile utensils (e.g., sterile gloves or bent glass rods).

E. Packaging of Product

When fish are packaged under special conditions such as VAC/MAP, a packaging scheme should be used which duplicates the condition of the product as it is normally packaged. Such products should be packaged in a manner that does not affect the normal course of changes within the package relative to the gas mixture. Alternative packaging may be used provided conditions within the package approximate the conditions within commercially packaged product.

F. Time and Temperatures of Incubation of Product

The recommended incubation temperatures for inoculated pack studies with nonproteolytic C. botulinum is 50°F. Incubation time should be one and one-half times the product's intended shelflife or up to the time when the product is overtly spoiled (unfit for human consumption).

G. Sample Size, Sampling Times, and Number of Samples to Test

Ideally, the entire sample should be homogenized or extracted for botulinal toxin testing. If samples are large (>300g), a minimum sample size of 50 g is recommended.

Sampling times should be adjusted according to the expected shelflife of the product. Samples for botulinal toxin assay should be taken at "0"-time (day of inoculation) and at a minimum of four additional times, with at least three sampling times between halfway and the final testing time.

The recommended minimum number of samples assayed for botulinal toxin at each sampling time is five. A minimum of three samples taken at "0"-time and at the final sampling time is recommended for C. botulinum enumeration. It is not necessary to continue sampling after two consecutive positive sampling times.

H. Botulinal Toxin Testing Procedure

The mouse bioassay procedure as described in the U.S. Food and Drug Administration Bacteriological Analytical Manual (6th ed., 1984) is the recommended method for botulinal toxin testing. Only individuals properly immunized with botulinal toxoid should perform these tests. Samples inoculated with nonproteolytic C. botulinum spores should be tested first without trypsinization. If mice survive, then tests should be done on trypsinized samples. Preferably, toxin analysis should be done on the day of sampling, but if this is not possible, samples should be homogenized and extracted in 0.05 M sodium phosphate-gel buffer (pH 6.2), sedimented by centrifugation (27,000 x g, 20 min, 4°C), and the supernatant fluids (at least 10 ml; adjusted to pH 6.2 if necessary) stored at refrigeration temperatures at or below 38°F(3.3°C), not to exceed 3 days.

An alternate procedure (such as an ELISA method) for the mouse bioassay test may be used providing the alternate method has been documented to be of equal or greater sensitivity than the mouse bioassay.

I. Product Analyses

In addition to botulinal toxin testing and C. botulinum spore enumeration, the product (duplicate samples) at "0"-time should be assayed for moisture, fat, pH, and aerobic plate count. Depending on the type of product, other analyses (such as protein, salt content, water activity, titratable acidity, nitrite content, sorbate content, psychrotroph count, spore count, lactic acid bacteria count, anaerobe count) also should be used. If the product is packaged under MAP, gas analysis should be done at the initial sampling time. Visual appearance (including gas formation [puffiness of samples]) and odor of samples should be determined and recorded at each sampling time. A sensory evaluation method using standard odor rejection criteria must be used.

APPENDIX 2

Statistical Evaluation

Stochastic modeling (also known as regression analysis, curve fitting, prediction modeling, forecasting, etc.) is extensively used in research and risk evaluation to predict the value of one criterion variable based on known (selected) values of one or more predictor variables. Stochastic models differ from mathematical (deterministic) models in that stochastic models are based on the sample data collected, whereas, mathematical models are derived from theory. In particular, stochastic models include an error estimation component while mathematical models do not.

It must be recognized that each stochastic model can be used for two different purposes, i.e., (1) to estimate the mean value of the distribution of the criterion variable which exists at each specified set of predictor variable values, and (2) to predict a single value in the distribution of the criterion variable at each specified set of predictor variable values. For example, in the fishery product shelflife studies, for each specific set of predictor variable values the stochastic model can be used to estimate the mean shelflife for all "packages" and to predict the shelflife for an individual package. The estimated mean and the predicted single value for toxin production can also be estimated for onset of toxin production. The estimated mean and the predicted single value for the criterion variable will have the same numerical value, however, the precision of the estimated mean as compared to the precision of the predicted single value can differ significantly.

Stochastic model building can be a very effective tool in research and risk evaluation. However, appropriate application of this methodology requires responsible recognition of potential limitations, particularly regarding the possible lack of sufficient precision in the obtained results for the intended purpose. It must be emphasized that predictions cannot be made beyond the limits of experimental data.

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